MDM2 SNP309 Is Associated with Endometrial Cancer Risk

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Abstract

Mouse double-minute 2 homologue (MDM2) is a key negative regulator of p53, a tumor suppressor gene that initiates cell cycle arrest and apoptosis in response to DNA damage and other cellular stresses. A T > G polymorphism found in the promoter region of MDM2 (SNP309) increases MDM2 expression and thereby attenuates p53 activity. We genotyped the MDM2 polymorphism SNP309 in endometrial cancer case-control studies nested within the Nurses’ Health Study (454 cases and 1,132 controls) and the Women’s Health Study (137 cases and 411 controls). Due to a significant difference in genotype distribution by ethnicity, we restricted our analyses to Caucasians. We calculated odds ratios and 95% confidence intervals using conditional and unconditional logistic regression adjusted for age at menarche, parity and age at first birth, postmenopausal hormone use at diagnosis, age at menopause and menopausal status at diagnosis, first-degree family history of colon cancer, body mass index at diagnosis, and cigarette smoking status at diagnosis. Women with a heterozygous genotype had no greater risk whereas those with a homozygous variant genotype had a greater risk than women with a wild-type genotype. In an effort to validate this finding, we evaluated the association between SNP309 and endometrial cancer risk among 73 cases and 79 controls (10), and reported a nearly 3-fold greater risk of endometrial cancer for women with a homozygous variant genotype. In an effort to validate this finding, we evaluated the association between SNP309 and endometrial cancer risk among 73 cases and 79 controls (10), and reported a nearly 3-fold greater risk of endometrial cancer for women with a homozygous variant genotype. In an effort to validate this finding, we evaluated the association between SNP309 and endometrial cancer risk among 73 cases and 79 controls (10), and reported a nearly 3-fold greater risk of endometrial cancer for women with a homozygous variant genotype. In an effort to validate this finding, we evaluated the association between SNP309 and endometrial cancer risk among 73 cases and 79 controls (10), and reported a nearly 3-fold greater risk of endometrial cancer for women with a homozygous variant genotype.

Introduction

Mouse double-minute 2 homologue (MDM2) is a key negative regulator of p53, a tumor suppressor gene that initiates cell cycle arrest and apoptosis in response to DNA damage and other cellular stresses (1). MDM2 suppresses p53 activity by ubiquitination and degradation (1). Overexpression of MDM2 is associated with accelerated cancer progression and lack of response to radiation or other DNA-damaging therapies (2).

In endometrial cancer tissue, p53 and MDM2 levels are correlated, suggesting that p53 is inactivated by MDM2 in endometrial cancer (3). Furthermore, Stewart and colleagues sequenced the TP53 gene in a series of endometrial cancer cases overexpressing p53 and found no mutations, suggesting that overexpression was due to another source such as MDM2 abnormalities (4).

For example, the Allele of the SNP309 variant accelerates tumor formation preferentially in women because it increases the affinity for Sp1, a cotranscriptional activator of the estrogen receptor (6). Women with the GG genotype were diagnosed with non–Hodgkin’s lymphoma or soft tissue sarcoma 13 to 14 years earlier on average than those with a TT genotype, although there was no appreciable difference in age at diagnosis for men (6). Similarly, among women homozygous for the SNP309 variant (GG), those with estrogen-sensitive breast cancers were diagnosed 7 years earlier than those with estrogen receptor–negative tumors (6).

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Materials and Methods

Nurses’ Health Study

Study Population. The Nurses’ Health Study (NHS) began in 1976 when 121,701 female registered nurses between the ages of 30 and 55 completed a self-administered questionnaire. Information regarding endometrial cancer risk factors was obtained from questionnaires completed every 2 years and at the time of blood collection. Dur-ing 1989 and 1990, blood samples were collected from women with pathologically confirmed invasive endometrial cancer except for nonmelanoma skin cancer. Controls were randomly selected participants with a blood sample and neither a hysterectomy nor diagnosed cancer except for nonmelanoma skin cancer. Cases diagnosed before June 1, 1998 who gave blood were matched to three controls, whereas cases diagnosed after June 1, 1998 who gave blood and all cases who gave cheek cells were matched to two controls. Matching factors included year of birth, menopausal status at specimen collection and at the cycle prior to diagnosis, and postmenopausal hormone use at the time of specimen collection (current versus not current users). For cases that gave blood, controls were also matched by time of day of blood collection, month of blood return, and fasting status at blood draw. For cases that gave cheek cells, controls were also matched on month and year of cheek cell collection. This case-control study consists of 454 endometrial cancer cases and 1,132 matched controls. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women’s Hospital, Boston, MA.

Women’s Health Study

Study Population. Begun in April 1993, the Women’s Health Study (WHS) is a completed randomized, double-blind, placebo-controlled trial investigating the benefits and risks of aspirin and vitamin E in the primary prevention of cancer and cardiovascular disease among 39,876 female health professionals, ages 45 years or older without a history of cancer (except nonmelanoma skin cancer), coronary heart disease, or cerebrovascular disease (12, 13). Written informed consent was obtained. A detailed description of the participants has been published previously (14). At the time of randomization, blood samples were obtained from 28,345 women (71%). Upon enrollment, all participants completed a detailed questionnaire including known or potential risk factors for endometrial cancer. Every 6 months for the first year, and annually thereafter, participants were sent follow-up questionnaires. Women were asked to report new diagnoses of major illnesses on their follow-up questionnaires; some women also provided this information through letters, or telephone calls. An Endpoints Com-mittee of physicians blinded to treatment reviewed records to confirm diagnoses. The trial was approved by the Institutional Review Board of Brigham and Women’s Hospital.

Case-control Study. Eligible WHS incident cases were women with pathologically confirmed invasive endometrial cancer diagnosed after blood collection (1993-1995) and before June 1, 2002, with no previously diagnosed cancer except for nonmelanoma skin cancer. Controls were randomly selected participants with a blood sample but with neither hysterectomy nor diagnosed cancer (except nonmelanoma skin cancer) at the time of blood draw. Controls were matched 3:1 to cases according to age at randomization, menopausal status, and postmenopausal hormone use at blood draw (current versus not current users). Controls were also matched to cases by date of blood return and fasting status at blood draw. This case-control study consisted of 137 incident endometrial cancer cases and 411 matched controls.

Genotyping. DNA was extracted from the buffy coat and cheek cell samples with the QIAGEN QIAmp Blood Kit (QIAGEN, Inc.). DNA extracted from NHS blood samples was whole genome–amplified with GE Health-care Genomiphi (GE Healthcare Bio-Sciences Corp.). We have previously shown that this method is robust for SNP genotyping (15).

MDM2 SNP309 (rs2279744) was genotyped by PCR amplification followed by restriction enzyme digestion with MspA1. Primers and conditions are available upon request. Replicate samples (5% of sample size) were included for quality control. Laboratory personnel were blinded to the location of quality control replicates and case/control status. Concordance of quality control replicates was 100%.

Table 1. Association between categorical MDM2 and endometrial cancer risk, NHS and WHS

<table>
<thead>
<tr>
<th>MDM2 SNP309</th>
<th>NHS Cases</th>
<th>NHS Controls</th>
<th>OR (95% CI) Adjusted*</th>
<th>WHS Cases</th>
<th>WHS Controls</th>
<th>OR (95% CI) Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>169 (43)</td>
<td>433 (46)</td>
<td>1.00</td>
<td>1.00</td>
<td>163 (44)</td>
<td>1.00</td>
</tr>
<tr>
<td>TG</td>
<td>162 (41)</td>
<td>420 (44)</td>
<td>0.98 (0.75-1.27)</td>
<td>0.95 (0.72-1.26)</td>
<td>54 (44)</td>
<td>1.22 (0.76-1.97)</td>
</tr>
<tr>
<td>GG</td>
<td>63 (16)</td>
<td>95 (10)</td>
<td>1.85 (1.26-2.72)</td>
<td>1.94 (1.26-3.00)</td>
<td>21 (17)</td>
<td>1.35 (0.74-2.47)</td>
</tr>
<tr>
<td>Test for trend</td>
<td>0.02</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for the matching factors and age at menarche (<12, 12, 13, >13 y), parity and age at first birth (nulliparous, parous with age at first birth <24 years, parous with age at first birth >24 years), first degree family history of colon cancer syndrome (yes, no), smoking status (never, former, current), body mass index (<25, 25-29, >30), age at menopause and menopausal status at diagnosis (premenopausal, postmenopausal with age at menopause <49, postmenopausal with age at menopause 49-51, postmenopausal with age at menopause >51, menopausal status unknown).
Table 1. Association between categorical MDM2 and endometrial cancer risk, NHS and WHS (Cont’d)

<table>
<thead>
<tr>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>Adjusted*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>216 (42)</td>
<td>596 (45)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>216 (42)</td>
<td>575 (44)</td>
<td>1.03 (0.82-1.29)</td>
<td>1.07 (0.74-1.55)</td>
</tr>
<tr>
<td>84 (16)</td>
<td>145 (11)</td>
<td>1.69 (1.22-2.34)</td>
<td>1.87 (1.29-2.73)</td>
</tr>
</tbody>
</table>

Statistical Analysis. Genotype frequencies were tested for Hardy-Weinberg equilibrium. Odds ratios (OR) and 95% confidence intervals (CI) were calculated using conditional logistic regression to assess the association between MDM2 genotypes and endometrial cancer risk. We evaluated a codominant model, a dominant model, and a trend in risk. Multivariate models were adjusted for age at menarche, parity and age at first birth, first-degree family history of colon cancer, smoking status at diagnosis, body mass index at diagnosis, age at menopause, and menopausal status at diagnosis, in addition to the matching factors. We used the DerSimonian and Laird random effects model to combine results from the two cohorts after testing for heterogeneity.

Women with a heterozygous genotype did not have a greater risk of endometrial cancer, but women with a homozgyous variant genotype had a greater risk than women with a wild-type SNP309 genotype (covariate-adjusted OR, 1.87; 95% CI, 1.29-2.73; Table 1). Mean age at diagnosis for women with the wild-type variant (59.6 years), heterozygous variant (60.1 years), or homozgyous variant genotypes (61.4 years) did not differ significantly (P = 0.21). In addition, the association between MDM2 SNP309 and endometrial cancer was not modified by cigarette smoking or postmenopausal hormone use.

Discussion

We evaluated dominant and additive models because a previous study showed a 2-fold increase in the MDM2 protein for cell lines with the heterozygous (TG) genotype and a 4-fold increase for cell lines with the homozygous variant (GG) genotype (5). Although the WHS results showed increasing risk with the number of variant alleles, the associations were not significant, most likely due to the small sample size. The NHS and pooled results showed no increase in risk for heterozygotes but a significantly increased risk for homozygous variants.

Walsh and colleagues found an increased risk of endometrial cancer with the homozygous variant genotype (OR, 2.76; 95% CI, 1.06-7.20) based on a small number of cases (n = 73) and controls (n = 79; ref. 10). Our observations are consistent with these results, although attenuated. Potential explanations for the differences in the strength of the association include sample size and population differences. The large CIs in the Walsh study (10) indicate unstable effect estimates due to small sample size. Twenty percent of their cases and 15% of their controls were non-Caucasian. Because the frequency of the SNP309 variant varies according to race (9), the association may have been overestimated due to confounding.

The study strengths include our homogenous population (which minimizes confounding by race), nested case-control design, and comprehensive disease follow-up. Additional studies in non-Caucasians are needed to evaluate the role of SNP309 in endometrial cancer risk for minority populations. To date, this is the largest study of the association between the SNP309 polymorphism in MDM2 and endometrial cancer risk.

If SNP309 imparts a survival advantage, there would be an overrepresentation of this polymorphism among prevalent cases, leading to a spurious association with endometrial cancer. However, we observed no difference
in the genotype distribution or association with disease when prevalent and incident cases were considered separately. In conclusion, the functional polymorphism in the MDM2 promoter, SNP309, may increase endometrial cancer risk.

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References
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